



Appl. No. 09/675,828

Ammdt. Dated June 29, 2004

Reply to Office Action of December 29, 2003 and Notice of Non-Compliant Amendment dated July 15, 2004

**AMENDMENTS TO THE CLAIMS:**

This listing of claims will replace all prior versions, and listings, of claims in the application:

Claims 1-27 (canceled)

Claim 28 (previously presented): The method of claim 33 wherein three or more target DNA's are amplified using a set of primers for each of said target DNA's, the primers in each of said primer sets having a  $T_m$  within the range of 65 to 74°C, all of said primer  $T_m$ 's being within about 5°C of each other, and said primers in each primer set having nucleotide lengths with differ from each other by no more than 5 nucleotides.

Claim 29 (previously presented): The method of claim 28 wherein each of said amplified target DNA's is captured with a capture reagent comprising a water-insoluble support to which is covalently attached a capture probe which is specific to a nucleic acid sequence of a strand of a distinct amplified target DNA strand, each capture probe having from 10 to 40 nucleotides and a  $T_m$  greater than about 50°C, and being hybridizable with said nucleic acid sequence of said distinct amplified target DNA strand at a temperature in the range of from 40 to 55°C.

Claims 30-32 (Canceled)

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Claim 33 (Previously presented): A method for the simultaneous amplification and subsequent simultaneous detection of a first target DNA and a second target DNA comprising:

A) simultaneously subjecting the denatured opposing strands of a first target DNA and the denatured opposing strands of a second target DNA to polymerase chain reaction in the presence of:

i) an aqueous composition buffered to a pH of from 7 to 9, and comprising, in the same solution: first and second primers which are specific to and hybridizable with, respectively, first and second nucleic acid sequences which are in opposing strands of a first target DNA and which are separated from each other along said opposing strands by from 90 to 400 nucleotides as measured 5' to 5', third and fourth primers which are specific to and hybridizable with, respectively, third and fourth nucleic acid sequences which are in opposing strands of a second target DNA which is the same as or different from said first target DNA, said third and fourth nucleic acid sequences being different from said first and second nucleic acid sequences and being separated from each other along said opposing strands of said second target DNA by from 90 to 400 nucleotides as measured 5' to 5', each of said first, second, third and fourth primers having a  $T_m$  within the range of from 65 to 74°C, as calculated by the formula  $T_m(^{\circ}\text{C}) = 67.5 + 0.34 (\% \text{ G} + \text{ C}) - 395/N$ , all of said primer  $T_m$ 's being within about 5°C of each other, said first and second primers having nucleotide lengths which differ

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from each other by no more than 5 nucleotides, and said third and fourth primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and

ii) the additional PCR reagents: a thermostable DNA polymerase, a DNA polymerase cofactor and dNTP's, any or all of said additional PCR reagents being supplied in the same or a different composition as defined in i), to simultaneously amplify said opposing first target DNA strands and said opposing second target DNA strands, provided that in each PCR cycle, each of priming and primer extension are carried out at a temperature within the range of from 62 to 75°C,

B) simultaneously detecting at least one of said amplified first target DNA strands and at least one of said amplified second target DNA strands as a simultaneous determination of the presence of said first and second target DNA's.

Claims 34-35 (Canceled)

Claim 36 (Previously presented): A method for the simultaneous amplification and subsequent simultaneous detection of a first target DNA and a second target DNA comprising:

A) simultaneously subjecting the denatured opposing strands of a first target DNA and the denatured opposing strands of a second target DNA to polymerase chain reaction in the presence of:

i) an aqueous composition buffered to a pH of from 7 to 9, and comprising, in the same solution:

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first and second primers which are specific to and hybridizable with, respectively, first and second nucleic acid sequences which are in opposing strands of a first target DNA and which are separated from each other along said opposing strands by from 90 to 400 nucleotides as measured 5' to 5',

third and fourth primers which are specific to and hybridizable with, respectively, third and fourth nucleic acid sequences which are in opposing strands of a second target DNA which is the same as or different from said first target DNA, said third and fourth nucleic acid sequences being different from said first and second nucleic acid sequences and being separated from each other along said opposing strands of said second target DNA by from 90 to 400 nucleotides as measured 5' to 5', each of said first, second, third and fourth primers having a  $T_m$  within the range of from 67 to 74°C, all of said primer  $T_m$ 's being within about 2°C of each other, said first and second primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and said third and fourth primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and

ii) the additional PCR reagents: a thermostable DNA polymerase, a DNA polymerase cofactor and dNTP's, any or all of said additional PCR reagents being supplied in the same or a different composition as defined in i), to simultaneously amplify said opposing first target DNA strands and said opposing second target DNA strands, provided that in each PCR cycle, priming and primer

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extension are carried out at the same temperature within the range of from 62 to 75°C,

B) simultaneously detecting at least one of said amplified first target DNA strands and at least one of said amplified second target DNA strands as a simultaneous determination of the presence of said first and second target DNA's.

Claim 37 (Currently amended): A method for the simultaneous amplification and subsequent simultaneous detection of a first target DNA and a second target DNA comprising:

A) simultaneously subjecting the denatured opposing strands of a first target DNA and the denatured opposing strands of a second target DNA to polymerase chain reaction in the presence of:

i) an aqueous composition buffered to a pH of from 7 to 9, and comprising, in the same solution: first and second primers which are specific to and hybridizable with, respectively, first and second nucleic acid sequences which are in opposing strands of a first target DNA and which are separated from each other along said opposing strands by from 90 to 400 nucleotides as measured 3' to 3', third and fourth primers which are specific to and hybridizable with, respectively, third and fourth nucleic acid sequences which are in opposing strands of a second target DNA which is the same as or different from said first target DNA, said third and fourth nucleic acid sequences being different from said first and second nucleic acid sequences and being separated from each other along said opposing strands of said second target DNA by

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from 90 to 400 nucleotides as measured [[or]] 3' to 3', each of said first, second, third and fourth primers having a  $T_m$  within the range of from 65 to 74°C, as calculated by the formula  $T_m(^{\circ}\text{C}) = 67.5 + 0.34 (\% \text{ G} + \text{ C}) - 395/N$ , all of said primer  $T_m$ 's being within about 5°C of each other, said first and second primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and said third and fourth primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and

ii) the additional PCR reagents: a thermostable DNA polymerase, a DNA polymerase cofactor and dNTP's, any or all of said additional PCR reagents being supplied in the same or a different composition as defined in i), to simultaneously amplify said opposing first target DNA strands and said opposing second target DNA strands, provided that in each PCR cycle, each of priming and primer extension are carried out at a temperature within the range of from 62 to 75°C,

B) simultaneously detecting at least one of said amplified first target DNA strands and at least one of said amplified second target DNA strands as a simultaneous determination of the presence of said first and second target DNA's.

Claim 38 (Previously presented): The method of claim 37 wherein three or more target DNA's are amplified using a set of primers for each of said target DNA's, the primers in each of said primer sets having a  $T_m$  within the range of

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65 to 74°C, all of said primer  $T_m$ 's being within about 5°C of each other, and said primers in each primer set having nucleotide lengths which differ from each other by no more than 5 nucleotides.

Claim 39 (Previously presented): The method of claim 38 wherein each of said amplified target DNA's is captured with a capture reagent comprising a water-insoluble support to which is covalently attached a capture probe which is specific to a nucleic acid sequence of a strand of a distinct amplified target DNA strand, each capture probe having from 10 to 40 nucleotides and a  $T_m$  greater than about 50°C, and being hybridizable with said nucleic acid sequence of said distinct amplified target DNA strand at a temperature in the range of from 40 to 55°C.

Claim 40 (Currently amended): A method for the simultaneous amplification and subsequent simultaneous detection of a first target DNA and a second target DNA comprising:

A) simultaneously subjecting the denatured opposing strands of a first target DNA and the denatured opposing strands of a second target DNA to polymerase chain reaction in the presence of:

i) an aqueous composition buffered to a pH of from 7 to 9, and comprising, in the same solution: first and second primers which are specific to and hybridizable with, respectively, first and second nucleic acid sequences which are in opposing strands of a first target DNA and which are separated from each other along

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said opposing strands by from 90 to 400 nucleotides as measured [[or]] 3' to 3',

third and fourth primers which are specific to and hybridizable with, respectively, third and fourth nucleic acid sequences which are in opposing strands of a second target DNA which is the same as or different from said first target DNA, said third and fourth nucleic acid sequences being different from said first and second nucleic acid sequences and being separated from each other along said opposing strands of said second target DNA by from 90 to 400 nucleotides as measured 3' to 3', each of said first, second, third and fourth primers having a  $T_m$  within the range of from 67 to 74°C, all of said primer  $T_m$ 's being within about 2°C of each other, said first and second primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and said third and fourth primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and

ii) the additional PCR reagents: a thermostable DNA polymerase, a DNA polymerase cofactor and dNTP's, any or all of said additional PCR reagents being supplied in the same or a different composition as defined in i), to simultaneously amplify said opposing first target DNA strands and said opposing second target DNA strands, provided that in each PCR cycle, priming and primer extension are carried out at the same temperature within the range of from 62 to 75°C,

B) simultaneously detecting at least one of said amplified first target DNA strands and at least one of said

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amplified second target DNA strands as a simultaneous determination of the presence of said first and second target DNA's.